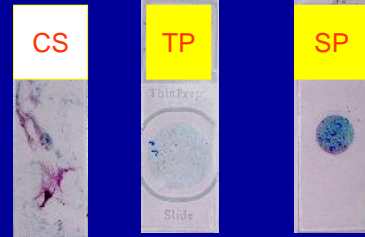


GYN Cytology

Fadi W. Abdul-Karim, MD MSMedu
 Department of Anatomic Pathology
 Vice Chair Education
 Professor of Pathology
 Cleveland Clinic
 Cleveland Ohio

Cervical Cytology Preparations



Conventional ThinPrep, 20mm SurePath, 13mm

Liquid Based Preparations

ThinPrep

Flatter prep/less dense cellularity

Bloody specimens
 Lubricant issues

Methanol fixative
 HC2 - FDA approved



SurePath

3 dimensionality/dense cellularity

Reduction of blood and inflammation

Ethanol fixative
 HC2-not FDA approved but validated



The 2001 Bethesda System

- Specimen Type
- Specimen Adequacy
- General Categorization (optional)
- Interpretation/Result
 - Negative for Intraepithelial Lesion or Malignancy (NILM)
 - Other
 - Epithelial Cell Abnormality
 - Other Malignant Neoplasms
- Ancillary Testing
- Automated Review
- Educational Notes and Suggestions (optional)

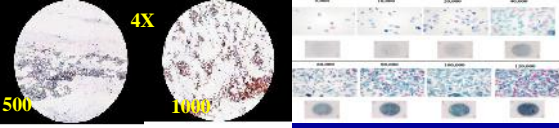
Specimen Adequacy Categories

- Satisfactory for evaluation:
 - Describe presence of TZ component and any other quality indicators (QIs) e.g. blood, inflammation
- Unsatisfactory for evaluation (specify reason...)
 - Specimen processed and examined, but unsat for evaluation of epithelial abnormality. CAP 50th % 1% CP/TP and 0.3% SP
 - Specimen rejected / not processed because of ...

TBS 2001

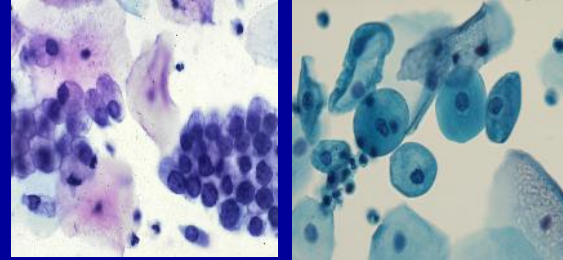
Definition of Cellularity

- Conventional Pap
 - 8-12,000 well-preserved & visualized squamous cells and squamous metaplastic cells
 - Range should be estimated
 - Use visual reference images (4x)
- Liquid Based Prep:
 - Minimum 5,000 squamous cells
 - Count 10 fields at 40X along center diameter
 - Imager /FOVs
 - Consider “holes” in overall cell count
 - Determine if limitation is technical in nature and reprocess if warranted



	BD SurePath	ThinPrep
# cells for 5K FN 20 eyepiece 10X	118.3	50
# cells for 5K FN 20 eyepiece 40X	7.4	3.1
# cells for 5K FN 22 eyepiece 10X	143.2	60.5
# cells for 5K FN 22 eyepiece 40X	9.0	3.8

10 well-preserved endocervical or squamous metaplastic cells singly or in clusters



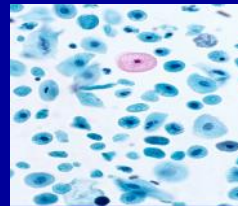
Glandular cells post hysterectomy (may be seen with fallopian tubes prolapse, endometriosis, fistula or adenosis)

Endocervical Component: Management

- **NILM, No ECC = Satisfactory Pap**
– 12 month follow-up
- **History abnormal pap, incomplete visualization of cervix, immunocompromised status, or poor screening history, repeat Pap in 6 months**

ACOG Practice Bulletin, Aug 2003

Endocervical Component: Atrophy

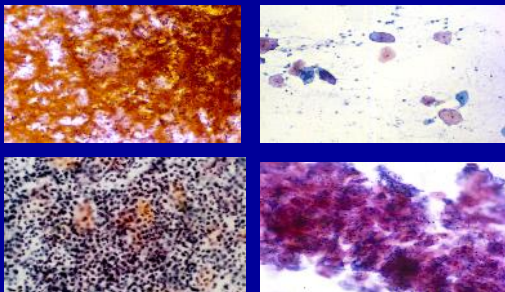


- Parabasal type cells may mimic squamous metaplasia and small columnar cells
- Post partum and progesterone may produce similar patterns as atrophy

- Only use squamous metaplasia as an ECC indicator if they can be definitively identified
- Comment on hormonal pattern inhibiting the ability to determine the presence of an ECC

Obscuring Factors: Quality Indicators

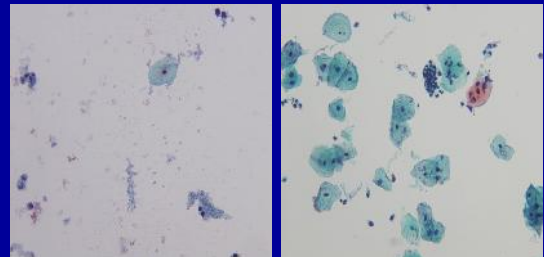
Satisfactory with QI 50% to 75% Unsatisfactory >75%



Reprocessing unsatisfactory TP specimens with GAA can substantially reduce the overall unsatisfactory rate and result in detection of significant lesion

TP Pap: Before GAA

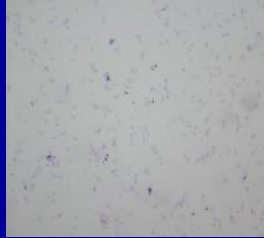
TP Pap: After GAA



TP Lubricant Use

lubricants that do not contain interfering substances:

- KY Jelly
- Surgilube
- Astroglide
- Crystelle



“Any specimen with abnormal cells is by definition satisfactory for evaluation.”

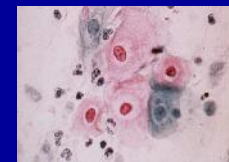
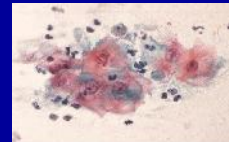
- If unsatisfactory, still:
 - Indicate organism, endometrials over 40 etc
 - May contain a higher rate of cancers or SIL on follow up specimens because of obscuring of a lesion by blood or inflammation.

The 2001 Bethesda System

- Specimen Type
- Specimen Adequacy
- General Categorization (optional)
- Interpretation/Result
 - NILM: Organisms/ Other Nonneoplastic Findings
 - Epithelial Cell Abnormality
 - Other Malignant Neoplasms
- Ancillary Testing
- Automated Review
- Educational Notes and Suggestions (optional)

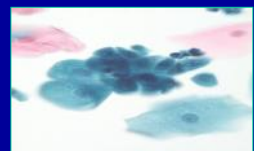
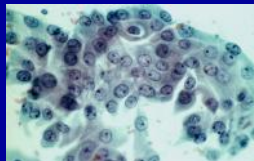
Reactive cellular changes associated with inflammation (includes typical repair)

- Bland nuclei > 1.5 to 2 X area of intermediate cell nucleus
- Bi and multi-nucleation.
- Round and uniform nuclei, smooth nuclear outlines.
- Mild hyperchromasia, even distribution.
- Nucleoli may be present
- Cytoplasmic polychromasia, vacuolization, halos



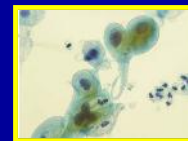
Reactive cellular changes associated with inflammation (includes typical repair)

- Flat, monolayer sheets
- Prominent nucleoli
- Nuclear enlargement
- Nuclear outlines are smooth and uniform
- Chromatin distribution is finely granular and regular



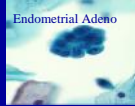
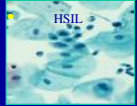
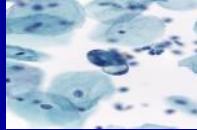
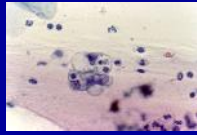
Reactive cellular changes associated with radiation/chemotherapy and FA deficiency

- Cytomegaly without an increase in N:C ratio
- Cytoplasmic vacuolization/polychromasia
- Coexisting changes of repair



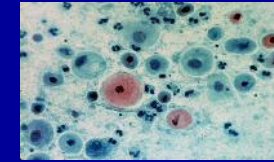
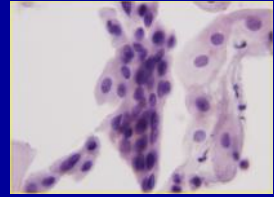
Reactive cellular changes associated with IUD

- Few glandular cells with large cytoplasmic vacuoles
- May have high N:C ratios and/or nucleoli
- Calcifications/ Actino.

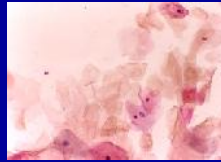
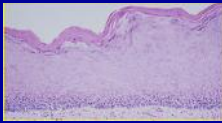


Atrophy

- Flat sheets of parabasal cells with preserved nuclear polarity
- Nuclei may be elongated with uniform chromatin distribution
- Giant cells.
- Dirty background
- Transitional metaplasia



Hyperkeratosis (HK) and Parakeratosis (PK)



Prolapse, inflamm, pessary, XRT

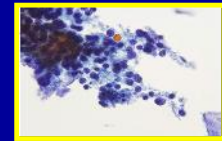
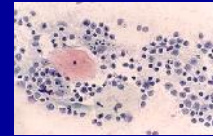
Little clinical significance when limited

May be seen overlying SIL
Abundant clusters may be significant
Follow closely



Follicular/Lymphocytic Cervicitis

- Polymorphous population of lymphocytes with or without tingible body macrophages
- Some are related to Chlamydia infection.



TBS: Epithelial Cell Abnormalities: Squamous cell

- **Atypical squamous cells (ASC)**
 - of undetermined significance (ASC-US)
 - cannot exclude HSIL (ASC-H)
- Low-grade squamous intraepithelial lesion (LSIL) encompassing: human papillomavirus/mild dysplasia/cervical intraepithelial neoplasia (CIN) 1
- High-grade squamous intraepithelial lesion (HSIL) encompassing: moderate and severe dysplasia, carcinoma in situ; CIN 2 and CIN 3
- Squamous cell carcinoma

Size relationships (Benchmarks)

- Cell size and shape
 - Position of nucleus
 - N:C ratio
 - Chromatin Pattern
 - Presence of nucleoli
 - Cytoplasm
 - Cell arrangements
 - Background information
- 35
- 50
- 70
- 100
- 150
- Intermediate cell
 - Squamous metaplasia
 - Reactive/ASC
 - ASC-US
 - SIL

Atypical Squamous Cells (ASC)

ASC-US: An ill-defined category created to address The inherent limitations of cytologic examination in the distinction of subtle cytologic changes

Atypical Squamous Cells (ASC): Cytologic changes suggestive of a squamous intraepithelial lesion that are quantitatively or qualitatively insufficient for a definitive interpretation.

Atypical Squamous Cells (ASC)

- The ASC category was developed to designate equivocal specimens not individual cells
- Does not represent a single biologic entity
- Subsumes changes unrelated to oncogenic HPV infection and neoplasia as well as those of SIL, and rarely carcinoma
- ASC may assume infinite appearances

ASC-US: Cytologic Features

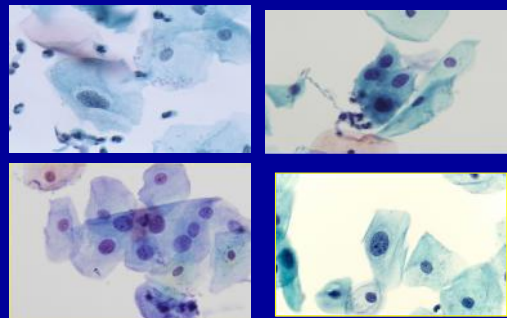
GENERAL:

- Cells are usually isolated
- Number of affected cells low (3-5)
- Cytoplasmic area approximate superficial or intermediate squamous cells in most instances and less often squamous metaplastic cells
- Absence of Human Papillomavirus (HPV) cytopathic effect

NUCLEAR:

- Area 2-3x size (2.5-3.5x) of intermediate cell nucleus or 2x nucleus of immature squamous metaplastic cell
- Limited variation in nuclear size & shape
- Nuclei are round and usually have smooth nuclear envelopes. Very limited nuclear membrane irregularity.
- Minimal nuclear hyperchromasia

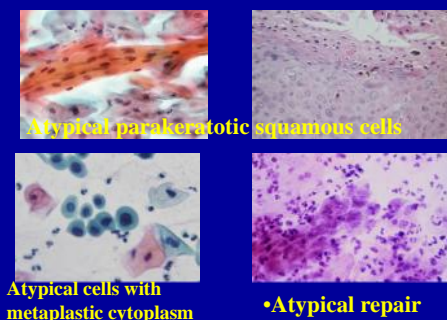
ASC-US: Cytologic Features



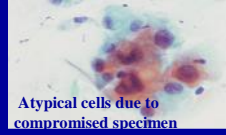
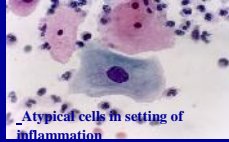
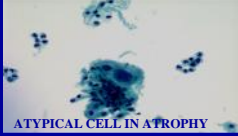
ASC-US: Not a Single Entity

- Atypical cells with “mature” intermediate type cytoplasm (NOS)
- Atypical parakeratotic squamous cells (AP)
- Atypical repair
- Atypical cells in the setting of atrophy
- Atypical cells due to compromised specimen

ASC-US: Not a Single Entity

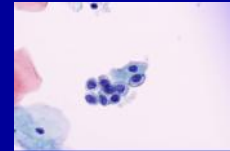
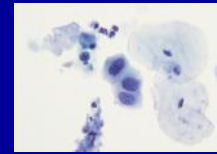


ASC-US: Not a Single Entity



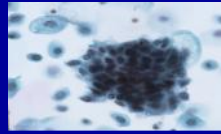
ASC-H: Changes that are suggestive of HSIL but lack criteria required for a definitive interpretation (5-10% of ASC)

- Sparse “atypical immature metaplastic cells”
- Singly or in tight small clusters (<10 cells)
- Slight increase in N/C ratio (usually 50%)
- Nuclear abnormalities
 - Mild irregular nuclear contours, mild hyperchromasia
 - Can be subtle

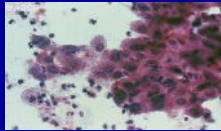


ASC-H

Crowded sheet pattern”



Severe atypical repair

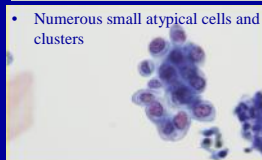
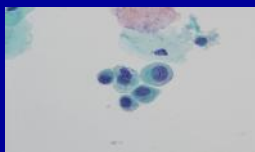
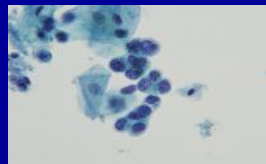
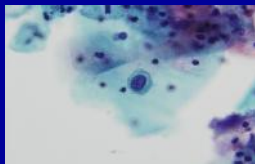


AS-H is not Synonymous with HSIL

- Not a “biologic” or “morphologic” entity.
- PPV for high grade lesion (CIN 2) is greater than ASC-US, but less than HSIL
- Should be investigated like HSIL, but if CIN2/3 is not confirmed by histology, may be spared aggressive treatment .
- Encourage pathologists to correlate cytology and histology if ↑ CIN2 is not confirmed.

ASC-H

HSIL: Increased N/C ratio (>75%). Irregular nuclear contours. Coarse chromatin



Sources of Histologic CIN 2+

- ASC-US: 4-5% of all cytologic interpretations and most common diagnosed cervical abnormality.
- Most common Pap interpretation that preceded a biopsy on CIN2+

ASC-US

- Few cells
- Slight nuclear enlargement (x2-3 intermediate cell nucleus)
- Stippled chromatin
- Regular nuclear membranes

LSIL

- Multiple cells
- Enlarged nucleus (>x3 intermediate cell nucleus)
- Smudgy or hyperchromatic nuclei
- Nuclear membrane irregularities
- Koilocytes

ASC-US < 5% of Paps
ASC/SIL ratio < 3.1 (1.4-2)

LSIL

GENERAL:

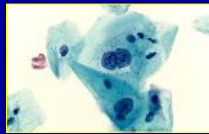
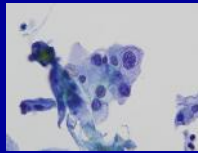
- Cells occur singly or in sheets
- Cytoplasmic maturation with distinct borders
- Koilocytes:
 - Perinuclear cavity: Sharply cut borders,
 - Various degrees of nuclear atypia /degeneration



LSIL

NUCLEUS:

- 3-6x size of ICN or 2-4x size of MCN
- Moderate variation in nuclear size
- Mild nuclear hyperchromasia
- Mild nuclear irregularities
- chromatin finely granular and uniformly distributed
- Nucleoli are absent
- Binucleation/multinucleation.



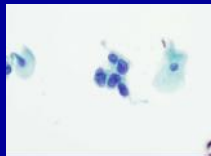
LSIL Cannot Rule Out HSIL (LSIL-H)

- Majority of cells are LSIL, few are ASC-H
- LSIL-H has slightly greater likelihood of underlying CIN-2 + (2 years) than LSIL (10.8%) and similar likelihood of CIN2/3+ as ASC- H (27%).
- Controversial as LSIL has built in a % of CIN2+ but
 - more sampling at colposcopy
 - deeper levels obtained by pathologist

HSIL

GENERAL:

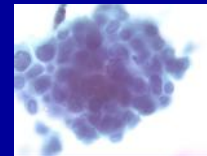
- Isolated cells and cell aggregates (syncytial-like arrangements)
- Immature cytoplasmic characteristics or pleomorphic keratinized configurations



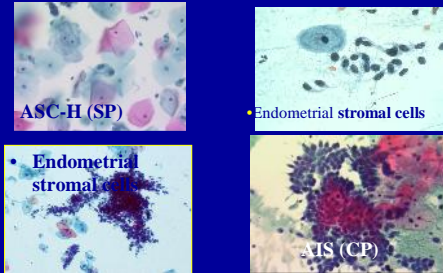
HSIL

NUCLEUS:

- Decreased nuclear size and marked increase in N/C ratio
- Nuclear hyperchromasia
- Chromatin clumping/coarsening of chromatin
- Irregularities of nuclear outlines



HSIL: Differential



HPV Prevalence

1. HR-HPV positive in:

- 95% HSIL/Carcinoma
 - 75-85% LSIL
 - 70-85% ASC-H
 - 50% ASCUS
 - Variable % normal Paps <10% (10-20%)
- HPV-16 and HPV-18 are associated with 70% of all invasive carcinomas.

HPV testing provides effective triage for ASC-US.

2. HR-HPV test captures virtually all CIN 2+:

- Cumulative probability of HSIL/CIN 2+ in 2 years ASC-US HPV positive is 25-30% (12-17% at incident colposcopy)
- The negative predictive value for the HPV test is 99 % (98.9-99.3%) .

HPV Testing and Management Guidelines

- HPV "Reflex" testing for equivocal ASC-US Cytology:
- LSIL is associated with HR-HPV and is not amenable to triage:
- Post colposcopy follow up of woman with abnormal cytology found to have less than a high grade lesion.

HPV Testing and Management Guidelines

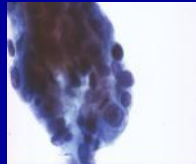
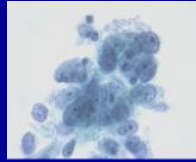
- Post treatment (LEEP or Cold knife cone) follow up to assess the risk of recurrence.
- As an adjunct to cytology in primary cervical cancer screening in 30y,
- Genotyping for HPV 16 and HPV18.

ASCCP: 2006 Guidelines(www.asccp.org)

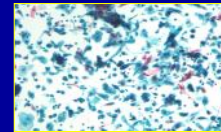
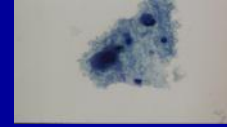
- Triage or follow up with HPV DNA testing is not recommended for ASC-US in adolescents.
- LSIL and ASC-US/HPV positive are managed similarly.
- ASC-H necessitates colposcopy/biopsy.
- For pregnant women the only indication for therapy is invasive cancer.
- HSIL necessitates colposcopy/biopsy.

Squamous Cell Carcinoma

- Greater depth-to-focus of cell groups
- Nuclear characteristics of malignancy
- Coarse irregular chromatin
- Nucleoli
- Irregular cytoplasmic shapes and conformations

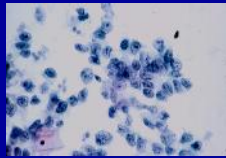


Squamous cell carcinoma: Tumor diathesis



Small Cell Carcinoma:

- Single cells and syncytial groups of small, cuboidal or round cells
- High N/C ratio with scant cyanophilic cytoplasm
- Large, round to oval nuclei with hyperchromatic, coarsely granular chromatin
- Nuclear molding and small nucleoli



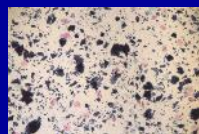
TBS: Epithelial Cell Abnormalities: Glandular cell

- Atypical
 - Endocervical cells (NOS or specify in comments)
 - Endocervical cells, favor neoplastic
 - Endometrial cells (NOS or specify in comments)
 - Glandular cells (NOS or specify in comments)
 - Glandular cells, favor neoplastic
- Endocervical adenocarcinoma *in situ*
- Adenocarcinoma
 - Endocervical
 - Endometrial
 - Extrauterine
 - Not otherwise specified (NOS)

Note: CIN is the most common significant finding identified in women with AGC. CIN lesions have been found in 8% to 83% of women with AGC, of which 40% to 68% are CIN 2,3.

Hyperchromatic crowded group: Grouping with features that impede the ability of the cytologist to see the individual cells in the middle

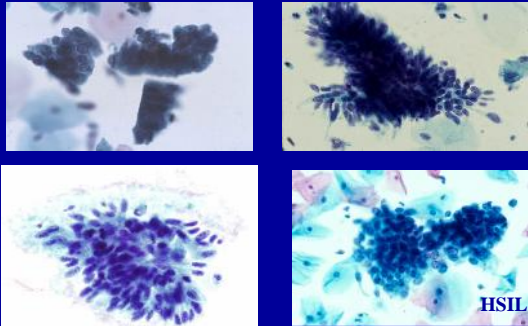
- Benign
 - Endocervical cells
 - Endometrial cells
 - LUS
 - Atrophy
 - Tubal metaplasia
 - Microglandular hyperplasia
 - Clusters of inflammatory cells
- Neoplastic/Preneoplastic
 - HSIL
 - AIS
 - Squamous cell carcinoma
- Adenocarcinomas



EC Adenocarcinoma in Situ (AIS)

- 1) Hyperchromatic crowded groups
- 2) Increased N/C ratio
- 3) Nuclei large (75 um²)
- 4) Even chromatin coarse granularity
- 5) Micronucleoli
- 6) Rosettes
- 7) Feathering
- 8) Strips with pseudostratification
- 9) Mitoses, apoptotic bodies
- 10) Amphophilic granular cytoplasm
- 11) Clean /inflammatory background

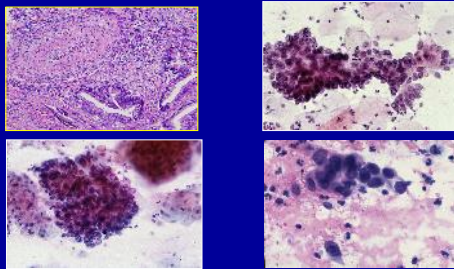
EC Adenocarcinoma in Situ



Endocervical Adenocarcinoma

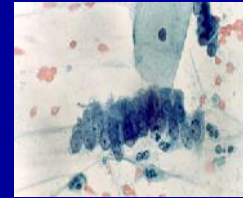
- 1) Abundant Cellularity
- 2) Flat or three D Sheets
- 3) Features of AIS
- 4) Single cells
- 5) Macro nucleoli
- 6) Decreased, finely vacuolated cytoplasm
- 7) Increased N/C ratio
- 8) Enlarged pleomorphic nuclei, nuclear irregularity, unevenly distributed chromatin/chromatin clearing.
- 10) Necrotic tumor diathesis

Endocervical Adenocarcinoma



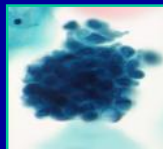
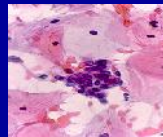
Tubal Metaplasia

- Pseudostratified, crowded groups of endocervical cells
- No nucleoli
- Cilia/terminal bars



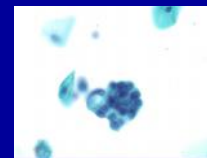
Endometrial Cells

- Report in all women 40 or older (0.5% to 1.8% of Paps).
- Tight 3-D clusters, loose clusters, or single cells. Bean-shaped nuclei. Nucleoli and chromatin pattern more apparent, Cytoplasmic vacuoles.

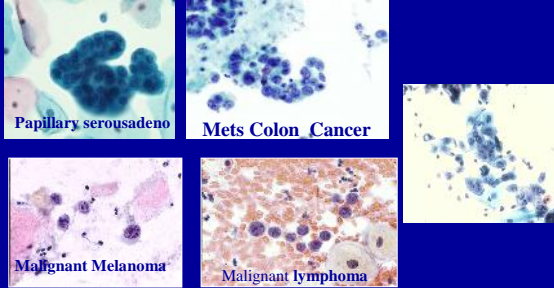


Endometrial Adenocarcinoma

- Single or small loose or tight clusters,
- Nuclear size increase with grade of the tumor,
- Variation in size and shape, unevenly distributed chromatin.
- nucleoli
- Small amount of vacuolated cytoplasm/intracytoplasmic neutrophils



Endometrial Adenocarcinoma and Metastatic Disease

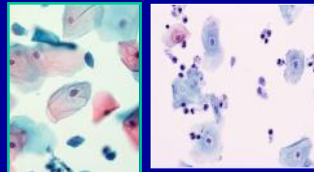


ASCCP: 2006 Guidelines(www.asccp.org)

- Colposcopy with endocervical sampling is recommended for all subcategories of AGC. Endometrial sampling is added for AGC >35 years .
- Cotesting for Paps > 30 years, guidelines for women (30 y and older) who test negative for HR-HPV and the role HPV-16 and 18 genotyping.

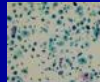
Trichomonas Vaginalis

- Pear-shaped (15-30 μm^2)
- Vesicular eosinophilic eccentric nucleus
- Cytoplasmic granules
- Dirty background.
- Flagella may be seen
- Cannonballs of inflammatory cells
- Pseudoeosinophilia, perinuclear halos and mildly enlarged nuclei.



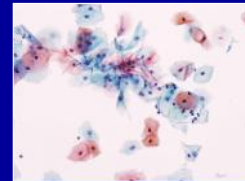
Mimickers::

- Cell fragments
- Cytoplasmic debris
- Stripped nuclei
- Degenerated polys
- Mucus blobs



Fungal organisms morphologically consistent with *Candida* spp.

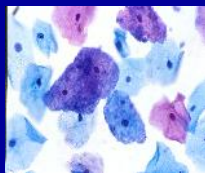
- Budding yeast
- 3-7 microns
- Pseudohyphae
- Fragmented polys and rouleau formation
- Spearing prominent pattern



- Mild nuclear enlargement and hyperchromasia
- Mimics ASCUS

Shift in flora suggestive of bacterial vaginosis (BV)

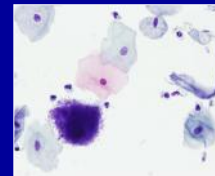
- Absence of lactobacilli
- Polymicrobial, as a synergistic infection with Gardnerella vaginalis and one or more anaerobic bacteria: Mobiluncus, Peptococcus or Bacteroides.
- Clue cells are fairly predictive of vaginosis



Clinical correlation (Vag pH, wet prep, “whiff” test on KOH preparation, cultures)

Bacteria morphologically consistent with *Actinomyces* spp.

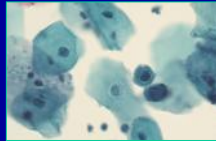
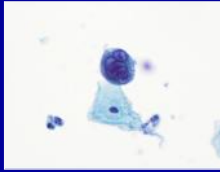
- Tangled clumps of filamentous organisms
- Acute angled branching
- Cotton-ball cluster
- Radial distribution of filaments.
- Acute inflammation.



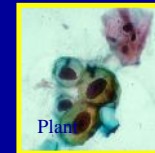
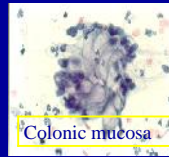
- Associated with IUD use
- May alert clinician to evidence of pelvic infection

Cellular changes consistent with herpes simplex virus

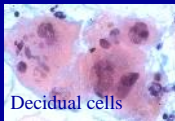
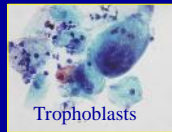
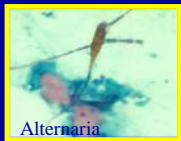
- “Ground-Glass” nuclei
- Dense eosinophilic intranuclear inclusion surrounded by a halo may be present
- Multinucleated epithelial cells with molded nuclei may be present.
- Single cells of herpes may mimic dysplasia



Artifacts



Additional Artifacts



Thank You